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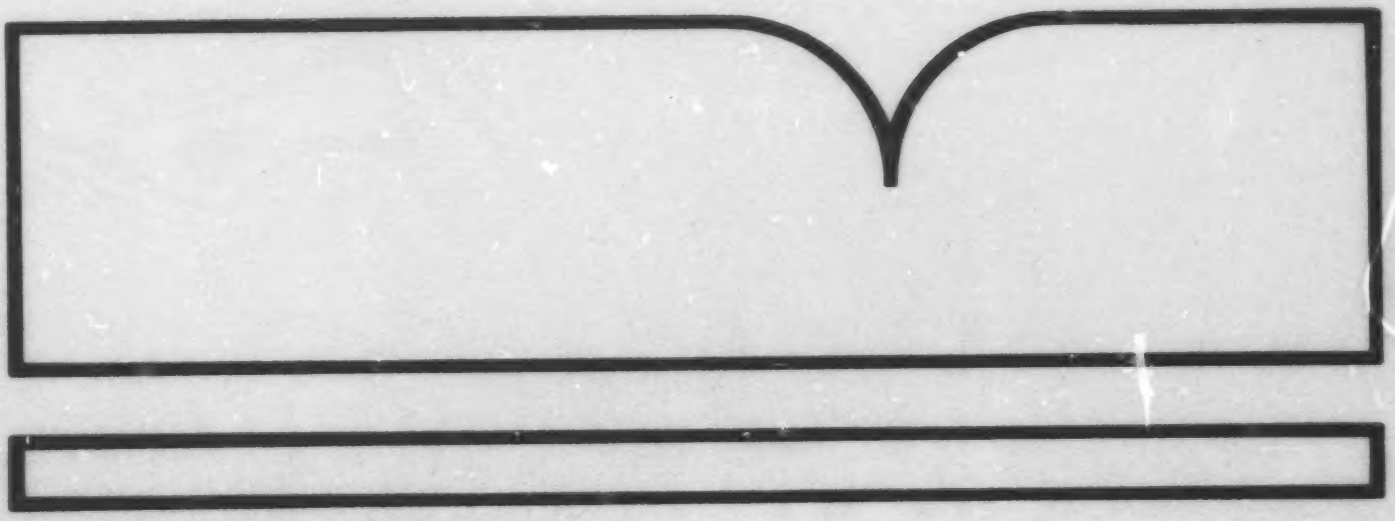

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16. ABSTRACT
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ACUTE AND CHRONIC EFFECTS OF HEAVY METALS AND CYANIDE ON *MYSIDOPSIS BAHIA* (CRUSTACEA:MYSIDACEA)

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Acute and whole life-cycle toxicity tests were conducted with the estuarine mysid shrimp, *Mysidopsis bahia*, exposed to cyanide and selected heavy metals. Acute toxicity values (96 h LC_{50}) ranged from 3.5 μg/l for mercury to 3130 μg/l for lead, and were ranked in order of toxicity: Hg > Cd > Cu > Cn > Ag > Zn > Ni > As > Cr > Pb. The chronic toxicity values ranged from 1.2 μg/l for mercury to 893 μg/l for arsenic. Chronic values were calculated from either survival, time to first reproduction, or number of young produced. When acute toxicity data for the same chemical are compared, *M. bahia* is consistently among the more sensitive marine species. Lack of comparable data precludes a similar observation with chronic tests. Examination of the relative sensitivity of the chronic responses indicates that only for cadmium was survival more sensitive than reproduction. Reproduction and survival were equally sensitive for mercury, zinc, nickel and arsenic, while reproduction was the most sensitive chronic response for the remaining four metals. The acute–chronic ratios calculated for *M. bahia* in this study ranged from 1.6 for cyanide to 125 for lead. Sixty percent of the acute chronic ratios were ≤5.5 and 90% ≤15.4.

Key words: mysid; sublethal effects; acute toxicity; reproduction; metals

INTRODUCTION

The extensive literature on the toxicity of heavy metals to marine organisms has been reviewed by Eisler et al. (1979). Examination of this data base reveals that the majority of results are for acute mortality studies from which 96 h LC_{50} values have been derived. Consequently, this type of toxicological data often formed the basis of environmental management decisions in the seventies (EPA, 1972, 1976). Mount (1968), Sprague (1976), and others recognized that this type of information was insufficient for predicting concentrations of pollutants that protect against chronic effects on an organism's growth and reproduction. The recent development of pollutant regulatory criteria for heavy metals and cyanide requires both acute and chronic toxicity data on the same species, collected under similar laboratory conditions (EPA, 1980).

The estuarine mysid, *Mysidopsis bahia*, was chosen by us as an acute and chronic test organism because of its demonstrated ecological importance as a food source for bottom feeding fish (Markle and Grant, 1970; Odum and Heald, 1972; Stickney

et al., 1974), and its amenability to laboratory culture (Nimmo et al., 1977).

The objectives of this study were: (1) to provide acute and chronic toxicity test data for *M. bahia* on cyanide and the heavy metals: zinc, silver, arsenic, copper, chromium and lead; (2) to compare the relative acute and chronic sensitivity of *M. bahia* to other species, (3) to determine the toxicity ranking of cyanide and selected heavy metals to *M. bahia*, and (4) to examine the relationship between concentrations producing acute and chronic toxicity for this species.

MATERIALS AND METHODS

Animals used in these tests were from our laboratory cultures. The original cultures of *M. bahia* were supplied by the EPA Environmental Research Laboratory at Gulf Breeze, Florida. The animals were acclimated from 23 ppt salinity to 30 ppt salinity by gradually adding filtered (15 μm) natural sea water over a five-day period so that salinity did not vary more than 2 ppt per 24 h. Sea water was heated to maintain the temperature at 23 $\pm$ 2°C.

Mysids were cultured for several generations in flow-through 76 l (20 gal) glass aquaria according to Gentile et al. (1982b). Cultures were continuously supplied with filtered (15 μm) natural sea water at a rate of 100 ml/min, resulting in a 99% volume exchange every 24 h. The outflows were covered by Nitex screen (250 μm) to prevent the escape of mysids or food organisms. Undergravel filters were used with a dolomite substrate one inch deep to maintain a pH of 7.8–8.2. The dolomite was covered by one inch of beach sand, the preferred substrate for these epibenthic animals. The sand was prewashed in deionized water and muffled at 500°F for 6 h to remove organic material.

Twenty-four hour post hatch nauplii of *Artemia salina* were added as food organisms at the rate of 7×10^4 nauplii/day for each 76 l culture (approximately 47 nauplii/mysid). This ration was adjusted according to mysid density; however, cultures were always fed ad libitum to prevent cannibalism of the young. Gentle aeration served to keep food in suspension and to provide a current conducive to feeding.

The continuous-flow toxicity tests were conducted in an exposure system described in Sosnowski et al. (1979). The exposure system employed a siphon-flush mechanism that produced a 50% exchange of volume every 4 h. The approximate flow rates were 30 ml/min inflow, and 100 ml/min outflow. Tests were conducted with filtered (15 μm) natural sea water at 30 $\pm$ 2 mg/kg salinity and temperatures ranging from 20–25°C depending on the compound tested. Within an individual toxicity test, the temperature ranged $\pm$ 1°C about the selected test temperature. Test systems were illuminated at 1000 lx on a 12L:12D cycle.

Acute and life-cycle tests were conducted with zinc, silver, arsenic, copper, chromium, lead, and cyanide. Primary atomic absorption standards were used as the toxicant sources in all tests. Toxicant solutions were prepared from these stan-

dards in deionized water at one hundred times the final desired concentration. The stock solutions were metered at a constant rate with a peristaltic pump, and proportionally diluted (1:100) with 15 μm filtered sea water immediately before entering the test chambers. The flow rate of dilution water was controlled with capillary tubing. Water samples from test chambers of the control and each concentration were analyzed twice weekly for the test metal (as total μg metal) with a Model 360* Perkin–Elmer atomic absorption spectrophotometer equipped with a Model 2100* Perkin–Elmer heated graphite atomizer (HGA-AA). Total cyanide determinations were conducted using a spectrophotometric analysis sensitive to 20 μg/l in sea water (U.S. EPA, 1979).

For both acute and life-cycle toxicity tests, 24-h-old post-larvae were randomly distributed to the exposure cups, five animals per cup. Six exposure cups were placed in each of the four toxicant concentrations and control. Mysids in each cup were fed 24 h posthatch *A. salina* (7×10^2 day^{-1}).

Acute 96 h toxicity tests were conducted according to ASTM Standard Methods (1980). Acute effects are reported as 96 h LC_{50} values (the estimated concentration at which 50% of the test organisms survived at 96 h); and are calculated by probit analysis or the moving average method (Stephan, 1977).

In the life cycle tests, the animals were exposed until sexually mature, then redistributed within each concentration to provide a consistent 2 male: 3 female ratio. The sublethal reproductive endpoints, time to sexual maturation, duration of embryonic development, and brood size, determined after 29–51 days of exposure, were quantified according to the methods of Gentile et al. (1982b). Effects of life-cycle tests are expressed as chronic values, the lowest concentrations having statistically decreased survival, delay of reproduction or decreased number of young produced per female reproductive day. Statistical differences from the control were calculated by analysis of variance and Dunnett's test (Snedecor and Cochran, 1967).

RESULTS

Acute toxicity data for cyanide and selected heavy metals with *M. bahia* from this and previous studies are ranked and summarized in Table I. Control survival in all 96 h tests was 100%. Among the inorganic compounds tested, mercury, LC_{50} = 3.5 μg/l, was the most toxic to *M. bahia* post-larvae, and lead, LC_{50} = 3130 μg/l was the least toxic. Cyanide, the only non-metallic inorganic tested, had an LC_{50} of 113 μg/l. The ratio of most to least toxic acute values is 894. However, mercury alone was 31 times more toxic than cadmium (LC_{50} = 110 μg/l) which ranked second in toxicity to juvenile *M. bahia*. Excluding mercury, the ratio of acute toxicity values for *M. bahia* was 30.

* Mention of commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

TABLE I

Summary of acute effects of nine metals and cyanide with *Mysidopsis bahia*. Tabluar values are μg/l with confidence intervals in parentheses.

Substance	96 h LC_{50}	Acute rank for *M. bahia*	Final acute value[a]	Acute rank *M. bahia*/ total taxa[a]
Mercury	3.5 (2.7–4.8)	1	3.8	1/24
Cadmium	110 (102–118)	2	75.4	1/26
Copper	181 (146–250)	3	6.4	13/17
Cyanide[b]	113 (96–137)	4	2.0	4/8
Silver	249 (220–283)	5	N.A	7/10
Zinc	499 (350–600)	6	173.0	7/24
Nickel	508 (387–635)	7	137.0	3/17
Arsenic	1740 (1390–2260)	8	242.3	2/9
Chromium	2030 (1560–2450)	9	2343.0	2/16
Lead	3130 (2350–INF)	10	434.3	7/10

[a] From USEPA ambient water quality criteria, 1980.
[b] Calculated with moving average method.
N.A. = not available.

The chronic effects of cyanide and the nine metals tested are presented in Table II. The relationship between exposure concentrations and the magnitude of the biological responses, and statistically significant differences for each, are noted. The range of chronic toxicity values, 1.2 μg/l for mercury to 893 μg/l for arsenic is similar to that reported for acute toxicity. Mercury (1.2 μg/l) and cadmium (7.1 μg/l) are the most chronically toxic, with silver (19.0 μg/l), lead (25 μg/l), copper (55 μg/l), cyanide (70 μg/l) and nickel (93 μg/l), representing intermediate toxicities. Lesser chronic toxicity was reported for chromium (132 μg/l), zinc (166 μg/l) and arsenic (893 μg/l).

The relative sensitivity of the types of chronic responses of *M. bahia* to life-cycle exposure was not consistent for all metals (Table III). The chronic values calculated for survival, time of first reproduction, or number of young produced were the same for mercury (1.2 μg/l), nickel (93 μg/l) and zinc (166 μg/l). Although effects on reproduction observed for mercury, nickel and zinc occurred at the same concentra-

TABLE II

The chronic effects of nine heavy metals and cyanide on *Mysidopsis bahia* in life-cycle toxicity tests.

Substance	Treatment[a] (µg/l)	Test duration (days)	Test temperature (°C)	Survival (%)	Days to first brood	Young/female reproductive day
Mercury[c,d] ($HgCl_2$)	CTL <0.05	35	21	80	25	0.34
	0.5 (± 0.2)	35	21	80	24	0.14
	0.8 (± 0.2)	35	21	60	26	0.30
	1.6 (± 0.5)	35	21	57[b]	35[b]	0.05[b]
	2.5 (± 0.5)	35	21	40[b]	42[b]	0.00[b]
Cadmium[e] ($CdCl_2$)	CTL <0.3	51	21	62	27	0.39
	5.1 (± 0.4)	51	21	83	23	0.26
	10.0 (± 1.0)	51	21	12[b]	23	0.66
	23.0 (± 5.0)	51	21	0.0[b]	–	–
	53.0 (± 7.0)	51	21	0.0[b]	–	–
Copper ($CuCl_2$)	CTL 2.9 (± 0.5)	35	21	47	26	0.32
	24 (± 7)	35	21	67	27	0.53
	38 (± 6)	35	21	60	26	0.33
	77 (± 7)	35	21	50	27	0.01[b]
	140 (± 12)	35	21	23[b]	–	0.00[b]
Silver ($AgNO_3$)	CTL <0.5	38	22	86	24	0.28
	3.0 (± 0.5)	38	22	88	25	0.27
	11 (± 1)	38	22	74	23	0.27
	32 (± 3)	38	22	79	25	0.16[b]
	108 (± 12)	38	22	57[b]	–	0.00[b]
Zinc ($ZnCl_2$)	CTL <2	36	21	73	21	0.71
	59 (± 8)	36	21	90	22	0.47
	120 (± 7)	36	21	80	21	0.81
	221 (± 16)	36	21	40[b]	24[b]	0.10[b]
	454 (± 32)	36	21	0.0[b]	–	–
Nickel[d] ($NiCl_2 \cdot 6H_2O$)	CTL <10	36	22	73	20	1.11
	30 (± 3)	36	22	80	20	0.79
	61 (± 6)	36	22	57	20	0.63
	141 (± 26)	36	22	30[b]	27[b]	0.33[b]
	297 (± 30)	36	22	0.0[b]	–	–
Arsenic ($NaAsO_2$)	CTL <3	36	23	80	22	0.51
	144 (± 17)	36	23	83	22	0.76
	303 (± 28)	36	23	40	22	0.53
	631 (± 27)	36	23	67	22	0.50
	1270 (± 73)	36	23	10[b]	23	0.03[b]
Chromium ($K_2Cr_2O_7$)	CTL <1	38	22	70	29	0.84
	88 (± 7)	38	22	57	26	1.10
	198 (± 18)	38	22	63	28	0.41[b]
	424 (± 25)	38	22	63	30	0.62[b]
	909 (± 68)	38	22	60	30	0.50[b]

TABLE II (continued)

Substance	Treatment[a] (μg/l)	Test duration (days)	Test temperature (°C)	Survival (%)	Days to first brood	Young/female reproductive day
Lead	CTL <2	44	21	80	28	0.61
($Pb(NO_2)_3$	17 (± 3)	44	21	97	26	0.44
	37 (± 6)	44	21	97	30	0.18[b]
	80 (± 12)	44	21	87	27	0.20[b]
	156 (± 14)	44	21	87	29	0.01[b]
Cyanide	CTL 0	29	24	97	18	0.42
(KCN)	<10	29	24	100	18	0.53
	11 (± 4)	29	24	87	18	0.58
	20 (± 4)	29	24	95	19	0.47
	43 (± 12)	29	24	90	18	0.32

[a] Seawater controls (CTL) averaged 30 ppt (range 28–32 ppt) and 22°C (range 20–25°C among all tests). Temperatures for each test ranged 1°C from the selected test temperature. Average measured concentrations were determined twice weekly during all tests.

[b] Statistically different from controls using ANOVA and Dunnett's test ($P < 0.05$).

[c] From Gentile et al., 1983.

[d] From Gentile et al., 1982a.

[e] From Gentile et al., 1982b.

tion as the effect on survival, there was no statistically significant decrease ($P < 0.05$) in the number of females available for reproduction (Gentile et al., 1982a, 1983). The effects on reproduction are therefore the result of reduced female productivity and not related to a decreased number of females available for reproduction.

Survival and number of young produced, were affected by silver, arsenic, copper and cadmium. Number of young produced was the most sensitive response for silver, copper and arsenic with chronic values of 19 μg/l, 55 μg/l, and 893 μg/l, respectively. For cadmium, however, survival was more sensitive than the number of young produced resulting in a chronic value of 7.1 μg/l (Gentile et al., 1982b).

The number of young produced was the only life-cycle parameter affected in tests with hexavalent chromium and lead. The chronic values for these metals were 132 μg/l and 25 μg/l, respectively. Only survival was affected during life-cycle exposure to cyanide resulting in a chronic value of 70 μg/l.

DISCUSSION

There have been several studies that rank the relative toxicity of heavy metals to marine species. Though not as comprehensive as that reported for *M. bahia*, the data permit a general assessment of the comparative toxicity of heavy metals. Brown and Ahsanullah (1971) found in acute and sub-lethal tests with the brine shrimp *A. salina* and the worm *Ophryotrocha labronica*, that the rank order of toxicity of the metals for the two species was very similar. Mercury and copper were highest in tox-

TABLE III

The chronic values with MATC limits in parentheses for *Mysidopsis bahia* exposed to nine heavy metals and cyanide. All tests were conducted under flow-through conditions with measured concentrations. Tabular values (in μg/l) are the lowest concentrations significantly different from control.

Substance	Chronic values		
	Survival[a]	Delay of reproduction[b]	Number of young produced[c]
Mercury[ef]	1.2 (0.8–1.6)	1.2 (0.8–1.6)	1.2 (0.8–1.6)
Nickel[f]	93 (61–141)	93 (61–141)	93 (61–141)
Zinc	166 (120–231)	166 (120–231)	166 (120–231)
Silver	59 (32–108)	> 32	19 (11–32)
Arsenic	893 (631–1270)	>1270	893 (631–1270)
Copper	104 (77–140)	> 77	55 (38–77)
Cadmium[dg]	7.1 (5.1–10.0)	> 10.0	15.2 (10.0–23.0)
Chromium	>909	> 909	132 (88–198)
Lead	>156	> 156	25 (17–37)
Cyanide	70 (43–113)	> 43	>43

[a] Number of first generation alive at end of test.
[b] Time until first brood release.
[c] Number of young produced per female reproductive day.
[d] Morphological abnormalities also occurred at 10.8 μg/l.
[e] From Gentile et al., 1983.
[f] From Gentile et al., 1982a.
[g] From Gentile et al., 1982b.

icity, lead was lowest, with cadmium, iron and zinc varying with the species tested. Watling (1982) found a differential rank for acute and sub-lethal effects with oysters. Based on growth measurements, the order of toxicity was Cu > Zn > Cd; for 96 h LC_{50} values the order as Cu > Cd > Zn. Our results also revealed a different rank for acute and sub-lethal effects for the metals over all, but in both cases Cd > Cu > Zn. Watling (1982) also reported a 96 h LC_{50} range of 45–80 μg/l mercury for oyster larvae which is an order of magnitude less sensitive than the mysid postlarvae at 3.5 μg/l. His study also determined a 96 h LC_{50} range of 40–75 μg/l

for cadmium with oysters, compared with our 96 h LC_{50} value of 110 μg/l for *M. bahia*.

Sub-lethal studies with metals, especially life-cycle tests, have been performed with comparatively few marine species. McKenney and Costlow (1981, 1982) found increased development time at 10 μg/l of mercury with the crab *Callinectes sapidus* and loss of adaptability to salinity and temperature with *Rhithropanopeus harrisii* at 5 mg/l. Reish et al. (1974) showed that 50 μg/l of zinc resulted in abnormal development of the polychaete, *Capitella capitata*, while Watling (1982) showed than 50–80 μg/l of zinc decreased the growth rate in three oyster species. The study reported here resulted in a chronic value for zinc of 166 μg/l for *M. bahia*.

In life-cycle studies with cadmium, we observed that the effect on the number of young produced was due to the fact that most females did not survive until reproductive age. Morphological abnormalities, an effect occurring only with cadmium, interfered with the normal molting process at the 10.0 μg/l resulting in the deaths of the test organisms (Gentile et al., 1982b). However, for the other metals, the effect on the number of young produced was not related to reduced survival (Table III). Similar effects have been noted with cadmium for both freshwater and marine organisms. Muramoto (1981) has found malformations occurring in the freshwater carp, *Cyprinus carpio* at 10 μg/l; and Marshall (1978) showed effects on the population biomass of *Daphnia galeata* at 7.7 μg/l. Biochemical effects on the lobster, *Homarus americanus* at 6 μg/l cadmium were demonstrated by Gould (1980); and reproductive effects on the estuarine copepod *Pseudodiaptomus coronatus* have been observed at 5 μg/l by Paffenhoffer and Knowles (1978).

In the marine environment, the effect of copper, chromium, and silver on growth and reproduction has been demonstrated for many organisms. Growth effects from copper have been observed for *M. edulis* at 10 μg/l (Calabrese et al., 1984) and *O. labronica* at 50 μg/l (Brown and Ahsanullah, 1971). Watling (1982) has shown an effect on growth rate at a range of 40–50 μg/l copper for three species of oyster. Winner and Farrell (1976) have shown effects of copper on the population growth rate of *Daphnia* sp. in fresh water at 60 μg/l. Our results show an effect on the number of young produced for *M. bahia* at 77 μg/l copper. Our studies with hexavalent chromium indicated only an effect on the number of young produced, with no delay of reproduction, at 198 μg/l. Mearns et al. (1976) and Oshida et al. (1981) found effects on the mean brood size and on the number of young produced in the polychaete *Neanthes arenaceodentata* at 12.5 μg/l of copper. Calabrese et al. (1984) found that silver affected growth of the mussel *M. edulis* at 25 μg/l which is consistent with our observations on reduction of the number of young produced by *M. bahia* at 19 μg/l.

The relationship between the concentrations of a toxicant producing acute and chronic biological effects is useful for developing margins of safety for untested species. However, the implicit assumption in this hypothesis is that the mode of toxicant action is the same for both acute and chronic responses among the species

under consideration. Accepting this assumption, the acute to chronic relationship for different species should be similar or vary in a predictable manner as a function of salinity or other water quality parameter. The available data, primarily for freshwater species, suggests that this hypothesis is supported for some chemicals and not for others (Mount, 1977).

Because of the lack of whole life-cycle chronic data for species other than *M. bahia*, the hypothesis cannot be adequately tested for marine species. Since the evidence from freshwater studies indicates the usefulness of this concept, further data for marine species should be produced to identify those types of toxicants for which the hypothesis is valid. The acute–chronic ratios calculated for *M. bahia* with the toxicants used in this study ranged from 1.6 for cyanide to 125 for lead (U.S. EPA, 1980). The acute–chronic ratios for the remaining eight compounds are mercury (2.9), copper (3.3), zinc (3.0), nickel (5.5), arsenic (1.9), cadmium (15.4), chromium (15.4) and silver (13.1). Sixty percent of the acute–chronic ratios for *M. bahia* are ≤ 5.5, and ninety percent ≤ 15.4, indicating a relatively narrow range between acute and chronic responses. It is important to determine the limits of applicability of the acute–chronic hypothesis for *M. bahia* because of its widespread use in toxicity testing and its importance in providing data for aquatic hazard assessment.

The following conclusions can be drawn from the results of this study. *M. bahia* is an excellent species for the conduct of chronic life-cycle tests because of its short life cycle, ease of handling, excellent long-term survival, sensitivity, and uniqueness of the manner of reproduction. The latter makes monitoring of the reproduction processes achievable and quantifiable under testing conditions. Acute and chronic toxicity data developed for this species are the most comprehensive for a marine invertebrate currently available. When acute toxicity data for the same chemical are compared, *M. bahia* tends to be among the more sensitive of the marine vertebrates and invertebrates tested. In examining the relative sensitivity of chronic responses, generalization is difficult. Only in the case of cadmium was survival a more sensitive response than reproduction. Reproduction and survival were of equal sensitivity to mercury, zinc, nickel and arsenic; and reproduction was the most sensitive response to copper, silver, chromium and lead. Defining the relationship between acute and chronic responses for *M. bahia* is important because the test data from this species is used in the regulatory process.

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